

WHAT IS CLAIMED IS:

1. A method of detecting an endocrine disrupting action of a test substance, comprising:

5 (1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which endocrine hormone and the test substance are present;

10 (2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first culture system with a second gene expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern.

15 2. A method of detecting an endocrine disrupting action of a test substance, comprising:

20 (1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which an endocrine hormone and the test substance are present;

(b) culturing the cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

25 (2) determining the presence or absence of an endocrine disrupting action of the test substance by obtaining a first gene expression pattern obtained from

the cell in the first culture system and a third gene expression pattern obtained from the cell in the third culture system, comparing the first gene expression pattern with the third gene expression pattern and a
5 second expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern.

3. A method of detecting an endocrine disrupting
10 action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

15 (b) culturing the cell a having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present;

(2) determining the presence or absence of an
20 endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first culture system with a third gene expression pattern obtained from the cell in the third culture system, thereby detecting a gene specific to
25 the first gene expression pattern.

4. A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

5 (b) culturing the cell a having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent; and

10 (c) culturing the cell a having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by
15 comparing a first gene expression pattern obtained from the cell in the first culture system with a second gene expression pattern obtained from the cell in the second culture system and the third gene expression pattern obtained from the cell in the third culture system,
20 thereby detecting a gene specific to the first gene expression pattern.

5. The method according to any one of claims 1 to 4, wherein said endocrine hormone is selected from the group consisting of estrogen, estradiol,
25 progesterone, androgen, testosterone, androsterone, cortisol, aldosterone, corticosterone, cortison, triiodothyronine, and tyroxacin; and said cell having

a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

5 6. The method according to any one of claims 1 to 4, wherein said endocrine hormone is triiodothyronine and said cell having a sensitivity to the endocrine hormone is Neuro2a.

10 7. The method according to any one of claims 1 to 4, wherein comparison of the gene expression patterns are made by comparing bands obtained by subjecting a gene group contained in each of gene expression patterns to electrophoretic separation.

15 8. The method of detecting an endocrine disrupting action of a test substance according to any one of claims 1 to 4, comprising

(a) recovering RNAs from each of the culture systems of (1);

(b) subjecting the RNAs recovered in the step (a) to reverse transcription;

20 (c) amplifying reverse transcription products obtained in (b) by PCR; and

(d) subjecting PCR products obtained in the step (c) to electrophoresis, comparing electrophoretic patterns of bands obtained, thereby detecting a band
25 specific to a first gene expression pattern.

9. The method according to any one of claims 1 to 4, wherein said gene expression patterns are

compared by hybridizing gene groups contained in each of the gene expression patterns with each other, and subtracting unhybridized genes.

10. A method of detecting an endocrine disrupting
5 action of a test substance, comprising:

(1) determining the presence or absence of an endocrine disrupting action of the test substance by

(a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which
10 the endocrine hormone and the test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which an endocrine hormone is present and test
15 substance is absent;

(c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(d) culturing a cell having a sensitivity to the endocrine hormone in a fourth culture system in which both the endocrine hormone and the test substance are absent;

(2) (a) isolating RNA from the first culture
25 system and preparing a first cDNA based on the RNA;

(b) isolating a second RNA from the second culture system;

(c) isolating a third RNA from the third culture system; and

(d) isolating RNA from the fourth culture system and preparing a fourth cDNA based on the RNA;

5 (3) (a) hybridizing the first cDNA and the second RNA and recovering unhybridized cDNA;

(b) hybridizing the third RNA and the fourth cDNA, recovering unhybridized RNA; and

10 (4) hybridizing the cDNA obtained in (a) of (3) and the RNA obtained in (b) of (3); and recovering unhybridized RNA, thereby detecting a gene specific to an endocrine disrupting action.

15 11. A polynucleotide obtained in a method comprising the following, and a complementary polynucleotide having a complementary sequence of said polynucleotide,

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and a test substance are present;

20 (b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent; and

25 (c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) obtaining first, second, third gene expression patterns from the first, second, third culture systems, and comparing the first, second, third gene expression patterns with each other, thereby
5 detecting and isolating a gene specific to the first gene expression pattern.

12. The polynucleotide and a complementary polynucleotide having a complementary sequence to said polynucleotide, according to claim 11,
10 wherein said endocrine hormone is selected from the group consisting of estrogen, estradiol, progesterone, androgen, testosterone, androsterone, cortisol, aldosterone, corticosterone, cortison, triiodothyronine, and tyroxacin; and said cell having
15 a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

13. A DNA chip comprising the polynucleotide and complementary polynucleotide according to claim 11 as
20 a nucleic acid probe.

14. A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system with the
25 endocrine hormone and the test substance; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by

comparing a first glycoprotein pattern obtained from the cell of the first culture system with a second glycoprotein pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a glycoprotein specific to the first glycoprotein pattern.

15. A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system with the endocrine hormone and the test substance; and

(b) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of endocrine disrupting action of the test substance by obtaining a first glycoprotein pattern obtained from the cell of the first culture system and a third glycoprotein pattern obtained from the cell of the third culture system, and

comparing the first glycoprotein pattern with the third glycoprotein pattern and a second glycoprotein pattern obtained from a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a glycoprotein specific to the first glycoprotein pattern.

16. A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present; and

(b) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of endocrine disrupting action of the test substance by comparing the first glycoprotein pattern obtained from the cell of the first culture system with the third glycoprotein pattern obtained from the cell of the third culture system, thereby detecting a glycoprotein specific to the first glycoprotein pattern.

17. A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test

substance is absent; and

(c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing first, second and third glycoprotein patterns obtained respectively from the cells of the first, second, third culture systems, thereby detecting a glycoprotein specific to the first glycoprotein pattern.

18. The method according to any one of claims 14 to 17, wherein said endocrine hormone is selected from the group consisting of estrogen, estradiol, progesterone, androgen, testosterone, androsterone, cortisol, aldosterone, corticosterone, cortison, triiodothyronine, and tyroxicin; and said cell having a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

19. The method according to any one of claims 14 to 17, wherein said endocrine hormone is triiodethyronine and said cell having a sensitivity to the endocrine hormone is Neuro2a.

20. The method according to any one of claims 14 to 17, wherein the glycoprotein specific to the first

culture system is performed by a method comprising:

(a) extracting proteins biosynthesized by a cell contained in each of culture systems of (1);

5 (b) recovering a glycoprotein by binding a substance capable of specifically binding to a polysaccharide chain contained in the proteins extracted in (a) to the polysaccharide chain;

10 (c) recovering a protein contained in the glycoprotein by cutting off the polysaccharide chain; subjecting the protein to electrophoresis, thereby obtaining a glycoprotein pattern for each of the culture systems; and

(d) comparing glycoprotein patterns to each other.

15 21. An antibody against a substance susceptible to an endocrine disrupting action, said antibody being prepared by a method comprising:

20 (1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and a test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent; and

25 (c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test

substance is present; and

(2) extracting proteins biosynthesized by a cell contained in each of the first, second and third culture systems of (1);

5 (3) recovering a glycoprotein by binding a substance capable of specifically binding to a polysaccharide chain contained in the proteins extracted in (2) to the polysaccharide chain;

10 (4) recovering a protein contained in the glycoprotein by cutting off the polysaccharide chain; subjecting the protein to electrophoresis, thereby obtaining a glycoprotein pattern for each of the first, second and third culture systems;

15 (5) comparing the glycoprotein patterns to each other, thereby detecting a glycoprotein specific to the first culture system, and further recovering a protein contained in the glycoprotein.

(6) preparing a polyclonal antibody against the protein obtained in (5),

20 (7) recovering a desired glycoprotein from the proteins extracted in (2) by the polyclonal antibody obtained; and

(8) preparing an antibody against to a polysaccharide chain contained in the glycoprotein
25 obtained in (7).

22. The antibody against the polysaccharide chain susceptible to an endocrine disrupting action,

according to claim 21, wherein said endocrine hormone is selected from the group consisting of estrogen, estradiol, progesterone, androgen, testosterone, androsterone, cortisol, aldosterone, corticosterone, cortison, triiodothyronine, and tyroxacin; and said
5 cell having a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

23. A protein chip comprising the antibody
10 according to claim 21.

2025 RELEASE UNDER E.O. 14176